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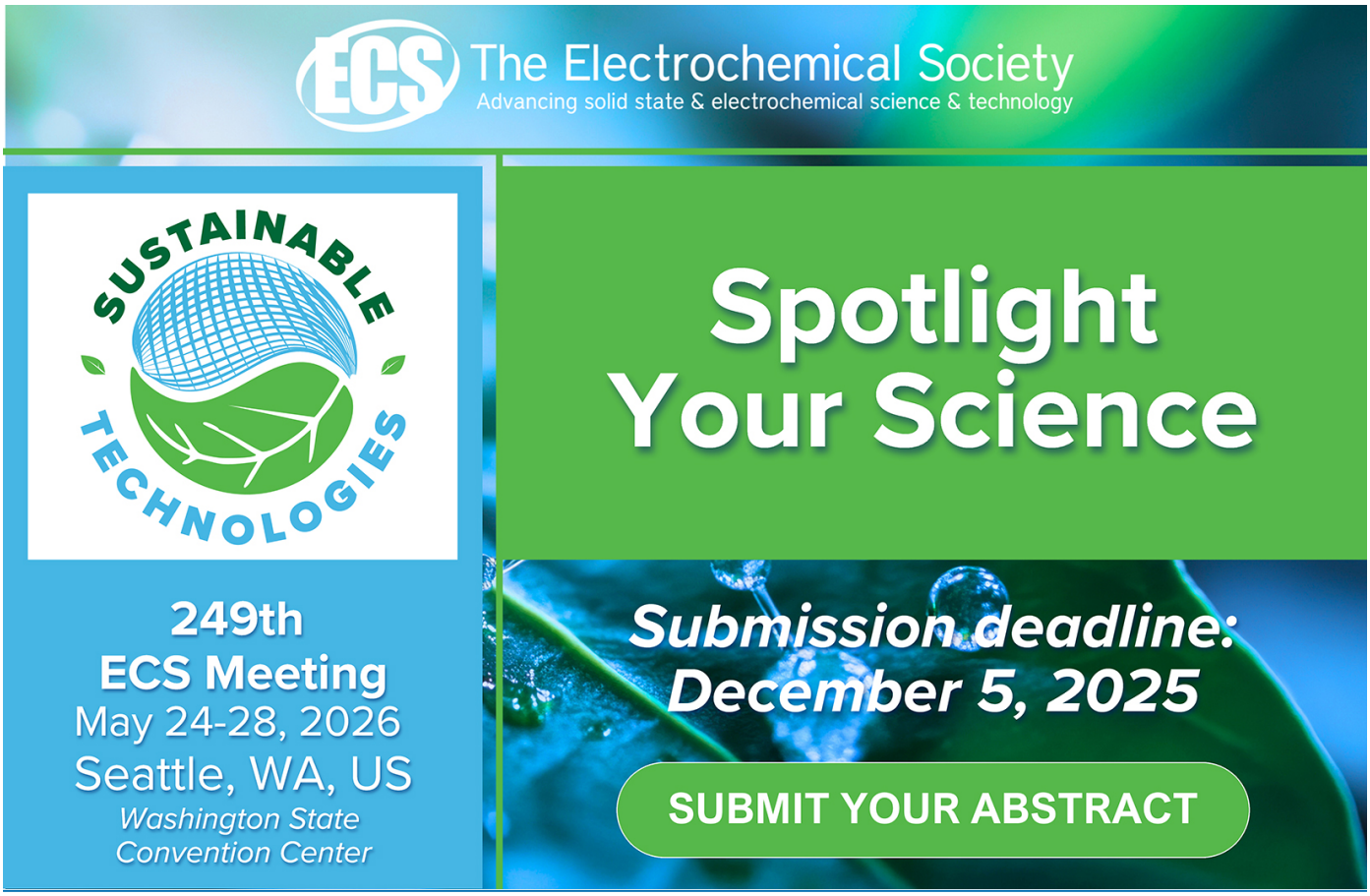

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Glucose Production from Sugarcane Bagasse by Two Stages Chemical Pretreatment & Hydrolysis

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Abstract. The aim of this work was to produce glucose from sugarcane waste. It consists of high cellulose, hemicellulose and lignin which enables to be converted into a glucose production. Three conditions of acid hydrolysis was measured by using One Factor at a Time (OFAT) which consist of sulphuric acid concentration, retention time and temperature. The highest yield and optimum conditions from OFAT parameters were further continue with the enzymatic hydrolysis by using Central Composite Design (CCD). The optimization of enzymatic hydrolysis for the conversion of cellulose to glucose was conducted by using three independent variables which were pH, temperature and enzyme dosage. Based on the data of Design of Expert (DOE) software from optimization of enzymatic hydrolysis, the optimum glucose production was determined by using Dinitrosalicylic Acid Reagent (DNS) method.

1. Introduction

For many years, increasing in interest was happened in a form of agricultural wastes as feedstock for biofuels production as it was considered in second generation. In Brazil, sugarcane was the major bioenergy crops where it used for the production of bioethanol. It is the most abundant co-products in the Brazilian sugar and ethanol industry where one ton of sugarcane was able to produce 280kg of bags [1]. Bagasse can be burned as a fuel about 50% to combined heat and power systems to meet energy which needs of the mills and the remainder is stored. Hence, the new development of technologies for ethanol production from sugarcane bagasse will increased the efficiency of ethanol production without expand the size of agricultural areas and can reduced the current conflict which effect the change in land use to meet growing energy demand [5].

Furthermore, sugarcane bagasse is a lignocellulose of a biomass. The cell wall mainly consists of three different components which were lignin, hemicellulose and cellulose. The high percentage of glucose and xylose from fraction of hemicellulose and cellulose in bagasse and the ability of microorganisms can convert the sugars into ethanol were the main factor of using this co-product for the biofuels production, one of the example of biofuel was ethanol production [2].

Sugarcane bagasse consist of mixture of hard fibre with smooth and soft parenchyma (pith) with high hygroscopic property and also consist of 35% moisture, it also have 60% of depithing efficiency and it will be removed about 20% as a pith either as a sugar or mill of pill premises of the production tonnage [5]. Thus, it have high tendency to produced bioethanol because of high carbohydrate contents which were 39.8% cellulose and 32.2% of hemicellulose.

In addition, for production of glucose it involved two steps which consist of chemical pretreatment and hydrolysis process. The removal of lignin and hemicellulose was done by pretreatment process of biomass to make the porosity of materials is reduced and also to reduce in

cellulose crystallization and rise. Fraction of hemicellulose in the biomass can be removed through process of dilute acid hydrolysis by using sulphuric acid as the catalyst [3]. To breaks down the lignin bond that exists in sugarcane bagasse, biomass pretreatment can be applied which can prevents the accessibility of cellulase enzyme. In addition, the hydrolysis of enzymatic rate can be enhanced by using the pretreatment process.

Then, the cellulose of enzymatic process is performed by higher specific of cellulase enzyme. The product that can be obtained after enzymatic hydrolysis is reducing sugar such as glucose. The features that influenced the cellulose of enzymatic hydrolysis of are temperature, retention time, pH, substrate concentration, enzyme dosage and also agitation speed [6].

2. Materials

2.1 Chemicals

Concentrated sulphuric acid (95%) was purchased from Fisher Pte Ltd, while for glucose, sodium citrate, citrate acid, potassium tartrate, Dinitrosalicylic acid reagent were purchased from Sigma Aldrich.

2.2 Plant materials

Sugarcane bagasse were collected at Taman Muhibbah, Perlis. The sugarcane bagasse was washed by using distilled water in order to remove dust and dry at 70°C overnight by putting the sugarcane bagasse into the oven. The dried sugar cane bagasse were ground into powder form and sieved by passing through a 1mm screen. Then, the sample were kept in a tightly closed container at room temperature to prevent contamination.

2.3 Procedures

2.3.1 Chemical preparation

The concentration of sulphuric acid (H2SO4) required in this pretreatment process was diluted sulphuric acid. The preparation of the solution by the dilution of concentrated sulphuric acid where the proportion of the concentrated acid is diluted with distilled water by using Equation 3.1.

$$V \times 1.84\ g/mL \times 0.96 = [(VF - V) + 1.84\ V]\ (\%\ of\ H2SO4) \quad (1)$$

Where VF = Final volume of diluted sulphuric acid
V =Volume of sulphuric acid in mL

The purity of the sulphuric acid were accounted in the dilution procedure.1.0% w/v of sulphuric acid were prepared from the concentrated sulphuric acid 96% w/v with the density of 1.84 g/mL by adding 5.69 mL of concentrated sulphuric acid solution into volumetric flask and dilute to 1.0% w/v with the final volume 1000mL with distilled water [4].

2.3.2 Citrate buffer

The citrate buffer were prepared from the stock solution of citric acid and conjugate base by mixing 10.5g of citric acid and 14.7g of sodium citrate in 500mL of distilled water respectively. Then, 82 mL of the citric acid solution were mixed with 18mL of sodium citrate solution in a volumetric flask. The total volume of the mixture were made up to 1 litre with distilled water. The solution were stirred with magnetic stirrer, 1M of sodium peroxide were added to adjust the pH to 4.5 with the help of a pH probe [4].

2.3.3 Dinitrosalicyclic Acid Reagent (DNS)

The DNS reagent were prepared by using conical flask with total volume of 250mL and the conical flask were wrapped with aluminium foil. First, 2.0g of sodium hydroxide were dissolved in 100mL of distilled water which produced 0.5mol/L sodium hydroxide solution. Then, the solution flask were

placed on a hot plate at 60°C and stirred for the entire preparation steps. Next, 75 g of potassium tartrate were added to the heated sodium hydroxide solution. After that, 2.5 g of 3, 5-dinitrosalicylic acid were added to mixture. The mixture then diluted to a final volume of 250ml by distilled water. The DNS solution were allowed to cool down to room temperature and kept in the refrigerator during the whole experiment [5].

2.3.4 Acid hydrolysis

For acid hydrolysis, 3.0 g sugarcane bagasse powder were added with 20mL of sulphuric acid in a 100mL conical flask, then the flask was wrapped with aluminium foil and heated in a water bath. The sample after the acid hydrolysis was filtered by the filter paper, the liquid fraction was tested by reducing sugar concentration.

For the first OFAT, the concentration of the sulphuric acid was the factor, then the temperature was set as 90°C and the retention time of 60 minutes in the water bath then the glucose concentration was tested using DNS method. The best sulphuric acid concentration is brought to next OFAT as a constant factor.

For the second OFAT, the retention time was the factor, then the sulphuric acid concentration based on the previous OFAT and the temperature was set to 90°C as constant in water bath then the reducing sugar concentration was tested. The best retention time is carry forward to the third OFAT as a constant factor.

For the third OFAT, the temperature is the factor, then the best concentration of sulphuric acid and retention time from the first and second OFAT was set as constant in a water bath then, the reducing sugar concentration was tested.

3. Results and Discussion

3.1 Acid Hydrolysis

One Factor At a Time (OFAT) was done by continuing manipulated three factors which were sulphuric acid concentration, retention time and temperature.

Figure 1 shows the effect of acid concentration on reducing sugar production (g/L). The first factor used to manipulate was the acid sulphuric concentration, the temperature and retention time were set as a constant which was 90°C and 60 minutes respectively. The highest reducing sugar concentration was 0.65 g/L produced at 0.5 % of sulphuric acid concentration. The reducing sugar concentration increased from 0 % to 0.5 % but when sulphuric acid concentration was between 1.25% and 3%, the reducing concentration decreased. This was because of formation of a xylose found as the main sugar in sugarcane bagasse of a hemicellulose hydrolysate[4].

Figure 2 shows the effect of retention time on reducing sugar concentration (g/L). 90 minutes produced the highest reducing sugar concentration which was 0.29 g/L. Based on the graph, after the optimum condition of the retention time, the increased value of retention time made the yield of reducing sugar concentration decreased because of the possibility of formation of other side products instead of glucose due to the extreme temperature. This is due to the highest chances that the pentose was not fully decomposed to furfural and disturbed the acid hydrolysis process [4].

Figure 3 shows the effect of temperature on reducing sugar concentration (g/L). It shows the increasing of reducing sugar production and 90°C was the optimum temperature which obtained 0.365 g/L of reducing sugar concentration as the sugarcane bagasse was decomposed by the sulphuric acid. However at 100°C it showed the decreasing of reducing sugar concentration. Glucose. The reducing sugar concentration was reduced because a portion of the sugar was degraded into enzyme-inhibiting by-products such as furfural (2-furaldehyde), 5-hydroxymethylfurfural (5-HMF), acetic acid, gypsum, vanillin, and aldehydes (4-hydroxybenzaldehyde, syringaldehyde) as a result of the conditions caused the cellulose to rapidly break into sugars [4].

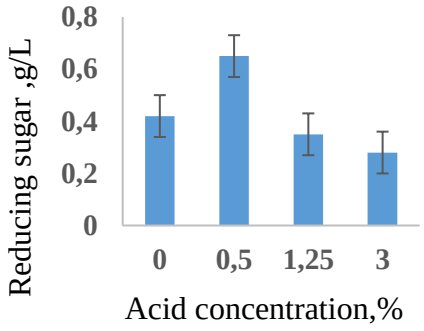

Figure 1. Effect of acid concentration on reducing sugar (g/L).

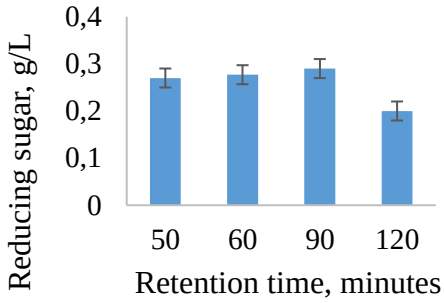

Figure 2. Effect of retention time on reducing sugar (g/L).

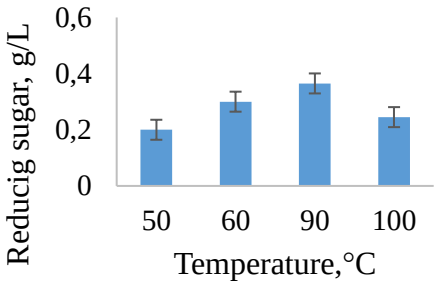

Figure 3. Effect of temperature on reducing sugar (g/L).

3.2 Optimization of Enzymatic Hydrolysis

The optimization of enzymatic hydrolysis of glucose production was conducted by using Design of Expert (DOE) on the Response Surface Methodology (RSM) via Central Composite Design (CCD) by using 5 level for each numeric factor. There were three independent variables selected to optimize the glucose production, which were temperature, pH and enzyme dosage by using cellulase enzyme while the response variables was glucose concentration. The effect on temperature, pH and enzyme dosage were determined in the range of 30°C to 50°C, pH 4 to pH 7 and 0.10mL to 0.20 mL respectively. The constant variables was agitation speed at 150 rpm. Based on Table 1, the optimum conditions for enzymatic hydrolysis was determined from the highest value of glucose concentration which at run 10, consists of pH 6.39, temperature 34°C and enzyme dosage 0.15mL.

Table 1. Design matrix of CCD and the experimental result.

Std	Run	Factor 1 : pH	Factor 2 : Temperature, (°C)	Factor 3 : Enzyme dosage, (mL)	Response: Glucose concentration, (g/L)
15	1	5.50	40.0	0.10	0.234
14	2	5.50	40.0	0.15	0.683
7	3	4.61	46.00	0.20	0.468
4	4	6.39	46.00	0.18	0.772
16	5	5.50	40.00	0.12	0.585
13	6	5.50	40.00	0.15	0.670
1	7	4.61	34.00	0.10	0.543
5	8	4.61	34.00	0.12	0.740
18	9	5.50	40.00	0.18	0.640
2	10	6.39	34.00	0.15	0.783
3	11	4.61	46.00	0.12	0.369
17	12	5.50	40.00	0.12	0.570
19	13	5.50	40.00	0.15	0.756
20	14	5.50	40.00	0.15	0.720
11	15	5.50	30.00	0.15	0.630
9	16	4.0	40.00	0.15	0.622
10	17	7.0	40.00	0.15	0.774
8	18	6.39	46.00	0.18	0.745
6	19	6.39	34.00	0.18	0.540
12	20	5.50	50.00	0.15	0.603

3.3 Regression Analysis

The result obtained from CCD were fitted in a quadratic or second-order equation to explain the dominance effect of substrate concentration and the enzyme dosage in terms of coded as A, B and C respectively. The purpose of the quadratic model was the mathematical explanation of the variables. The final empirical models in terms of coded and actual factors were shown in the following equation:

Final equation in terms of coded factors:
Glucose Concentration
$= 0.73 + 0.038A - 0.021B + 0.024C - 0.036AB + 0.061AC + 0.17BC - 8.401E\text{-}003A^2 - 0.037B2 - 0.15C^2$

Where the alphabetical was represents as:
A: pH
B: Temperature
C: Enzyme dosage

Final equation in terms of actual factors:
Glucose concentration
= 1.01252 + 0.087903 pH - 0.029585 temperature – 0.56996 enzyme dosage – 0.0105612 pH – 1.052622 temperature – 168.778722 enzyme dosage

3.3.1 Analysis of Variance (ANOVA)

The analysis of variance (ANOVA) was a series of statistical hypothesis testing which was used to investigate whether statistical relationship between the means of independent variables. To determine the model satisfies the assumption of ANOVA, which was shown in Table 2 and Table 3.

Table 2. Analysis of Variance (ANOVA) for surface quadratic model.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.38	9	0.042	27.39	< 0.0001	Significant
A-pH	0.014	1	0.014	9.33	0.0122	
B-temperature	4.866E-003	1	4.866E-003	3.20	0.1040	
C-enzyme dosage	4.860E-003	1	4.860E-003	3.20	0.1041	
AB	2.392E-003	1	2.392E-003	1.57	0.2384	
AC	0.013	1	0.013	8.80	0.0141	
BC	0.061	1	0.061	39.94	< 0.0001	
A^2	8.222E-004	1	8.222E-004	0.54	0.4791	
B^2	0.016	1	0.016	10.61	0.0086	
C^2	0.17	1	0.17	111.05	< 0.0001	
Residual	0.015	10	1.521E-003			
Lack of Fit	0.011	6	1.764E-003	1.52	0.3557	Not significant
Pure Error	4.627E-003	4	1.157E-003			
Cor Total	0.39	19				

Table 3. R-Squared value for model developed from CCD.

Parameters	Value
Standard Deviation	0.039
Mean	0.62
C.V%	6.27
R-Squared	0.9610
Adjusted R-Squared	0.9259
Predicted R-Squared	0.8493
Adequate Precision	18.517

3.3.2 Graphical Analysis

In the previous discussed, regression analysis was the residual difference between the observed value of the dependent variable and predicted variable. The normal plot with residue and the plots of residual versus predicted were analysed as shown in Figure 4 and Figure 5.

In the normal probability plot of the raw data, the analysis of variance shown a straightforward relationship with the residual. Figure 4 was the quadratic polynomial model satisfies the assumptions analysis of variance (ANOVA) was near to the normal. If the model was correct and the assumption was satisfied, the residual might without structure. In fact, they should be no relation to any other variable including plotting the residuals versus the fitted predicted response. A simple check would include plotting the residuals versus the fitted predicted values. A plot of residuals versus the rising

predicted response value test the assumption of the constant variance. Based on figure 5, the plot was a random scatter which was justified the need for no alternation to minimize personal error.

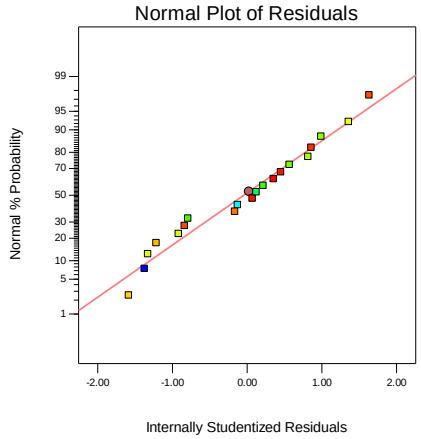

Figure 4. Normal probability plot of standardized residuals.

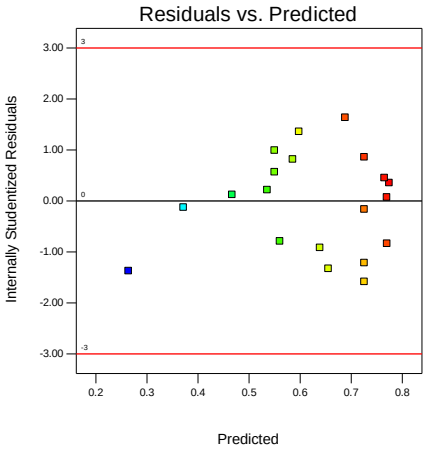

Figure 5. Studentized residual versus predicted values.

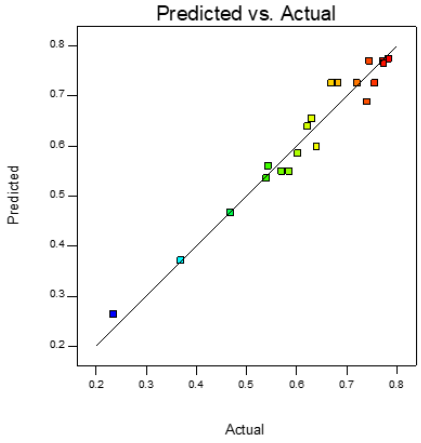

Figure 6. Predicted versus actual glucose concentration.

Figure 6 shows the predicted versus actual glucose concentration graph. The predicted versus the actual plot reveals how well the models can be fit into the data. The actual data means the

experimental data from the experiment where the predicted data was evaluated by the DOE software. Based on Figure 6, there was a distance results between the actual and predicted data. Points was a distant from the straight line indicate that the existence of outliers. The result improved in order to fit well in the quadratic model.

3.3.3 Effects of temperature, pH and enzyme Dosting

The response surface was used to obtain the maximum response which was the total glucose concentration at the optimum conditions at each design variables. Variables temperature, pH and enzyme dosing were considered in this study for the interaction and the effect the response variable which was the glucose concentration produced.

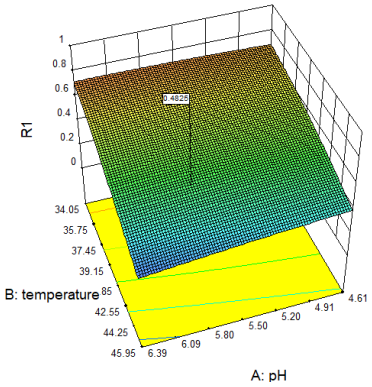

Figure 7. 3D plot interaction between temperature, pH and enzyme dosing.

The two dimensional (2D) contour plot and three dimensional (3D) response surface were used as a graphical tool to display the interaction between the temperature, pH and enzyme dosing on glucose production. Figure 7 and figure 8 shown the relationship between three variables. Figure 7 demonstrated that a surface with a maximum point located inside the experimental region. Figure 8 shown a flat shape contour plot with the related variables were vital.

Based on the 2D and 3D figures shown the glucose increased when the temperature increased but it started to decline when the temperature and pH after the optimum value. Temperature and pH were main factors governing the saccharification. The variation in temperature and pH activity in the enzyme cellulase might due to the active side chain that were fully occupied at this concentration and thus produced the optimum glucose. Temperature and pH value must be optimized to indicate the good hydrolysis on the specified maximum concentration leading to high glucose yield and decreases overall cost.

In addition, the effect of enzyme dosing was a plot in the range of 0.10ml to 0.20ml, thus the optimum result was 0.15ml. Biomass which required the lower enzyme dosage must be utilized to continuous development in order to decrease the cost of enzyme.

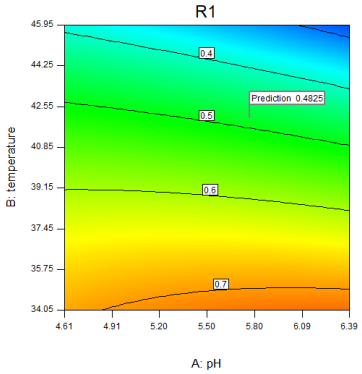

Figure 8. 2D of Contour plot of interaction between temperature, pH and enzyme dosage.

4. Conclusion

The sugarcane bagasse was a suitable raw material for the glucose production. Method in One Factor at a Time (OFAT) was able to determine the best conditions of acid hydrolysis which consisted of three parameters, namely acid concentration, retention time and temperature which were 0.5 %, 90 minutes and 90°C respectively. By using Surface Response Methodology (RSM) through Central Composite Design (CCD), the optimum conditions between the relationship of temperature, pH, and enzyme dosage were carried out by 20 experiments for glucose concentration determination. The experimental results showed that at optimum parameters of 34°C, pH of 6.39 and 0.15mL of enzyme dosage produced 0.783 g/L of glucose. Predicted R-Squared in Analysis of variance (ANOVA) correlation coefficients was 0.8493 and adjusted R-Squared was 0.9259 indicated moderate of the experimental data evaluation by a quadratic regression model. The determination of the coefficient of R-Squared was 0.9610 which corresponds to the standard deviation of 0.039. The magnitude of the R-Squared indicated there was a close relationship between the value of actual and also with the predicted value and the value for model F was 27.39. Last but not least the high yield of glucose production was 0.783 g/L.

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